

CLAIMS

1. A method for isolating progenitor cells from a human body, inclusive of all cells with stem cell-like characteristics, wherein such cells are directly or indirectly derived from human mammary secretion, be it colostrum, mature milk, or dry
5 period secretion from males or females, of said human body during at least one of the following periods: non-pregnant period, pregnant period, lactating period, involuting period.
2. A method according to claim 1, wherein the progenitor cells are pluripotent or
10 multipotent.
3. A method according to claim 1, wherein said progenitor cells are isolated from the mammary secretion in that noncellular parts of the mammary secretion are separated from the cellular parts.
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4. A method according to claim 3, wherein non-pluripotent or non-multipotent cells are removed from cellular parts.
5. A method according to any of the preceding claims, wherein secretory epithelial
20 cells, leucocytes and in particular nonhuman cells like bacterial cells are removed from the mammary secretion.
6. A method according to any of the preceding claims, wherein the mammary secretion during lactating periods is used for the isolation of the progenitor cells,
25 and wherein the mammary secretion during particular stages of mammary secretion such as: after beginning of individual feeding; versus end of individual feeding; lactation phase; preferably early lactation.

7. A method according to any of the preceding claims, wherein magnet beads are used to the isolation of the progenitor cells.
- 5 8. A method according to any of the preceding claims, wherein in a first step cellular components are washed out of the mammary secretion, in a second step said cellular components are stained with antibodies to the progenitor cell markers, and in a third step the progenitor cells are separated from the other cells directly or indirectly by means of the attached antibodies.
- 10 9. A method according to claim 8, wherein the antibody-stained progenitor cells are attached to beads, preferably small iron beads, and wherein the progenitor cells are extracted by means of the beads, preferably in case of small iron beads by using a magnet, and wherein subsequently the beads as well as if need be the antibodies are removed from the progenitor cells.
- 15 10. A method according to claim 9, wherein removal of the beads is effected by means of enzymes selected from the following group: Dnase, Proteinase, Rnase.
- 20 11. A method according to any of the preceding claims, wherein the progenitor cells are cultured without using a fibroblast feeder layer, in particular without using a mouse fibroblast feeder layer.
- 25 12. A method according to any of the preceding claims, wherein in
(i) a first step the whole human mammary secretion is subjected to centrifugation leaving a fat layer on top, a protein and carbohydrate rich supernatant beneath it, and at the bottom a pellet of cells;

- (ii) in a second step fat fraction and supernatant are removed;
- (iii) in a third step a buffer, such as, but not limited to, phosphate buffered saline, tris buffer saline, TBS and/or PBS, or media, such as, but not limited to, Williams media or RPMI Media, is added and the cells are resuspended in the buffer / media and centrifuged as before, preferentially repeating this process 3 or 4 times, leaving a substantially pure cell pellet;
- (iv) and in a fourth step the progenitor cells are separated from the cell pellet.

13. A method according to any preceding claim, wherein a cell pellet is generated from the human mammary secretion, and subsequently the following separation steps are used:

(v) the cell pellet is suspended in media, preferentially in RPMI media containing foetal calf (bovine) serum,

(vi) this suspension is incubated with magnetic beads which have before been incubated with progenitor, preferentially stem cell-specific antibodies, like anti-mouse IgG antibodies, which antibodies are attached to the magnetic beads via a small strand of DNA, wherein the incubation of the cell suspension is preferentially carried out for 15 minutes at 4°C;

(vii) once the progenitor cells have bound to the magnetic beads a magnet is attached to the tube containing the cells/beads, thus attracting the progenitor cells connected with the beads to the magnet, whereas unbound cells are not and remain in the supernatant;

(viii) removing the supernatant leaving only the progenitor cells bound to the beads via the progenitor cell antibody.

14. A method according to claim 13, wherein subsequently, the following steps are used:

(ix) progenitor cells bound to the beads via the stem cell antibody are removed by an appropriate cleavage means, preferentially, in case of the antibody being

attached to the beads via small strand of DNA, a by means of addition of a Dnase,

(x) the beads are removed by attaching the magnet once more such that the beads, no longer attached to the stem cells, are attracted to it;

(xi) removing the supernatant now containing the isolated progenitor cells.

15. A method according to any of claims 1-12, wherein the cells are separated from human mammary secretion by centrifugation, subsequently incubated in a growth media that is permissive for progenitor/stem cell/lactocyte growth.

16. A method according to claim 15, wherein in

(i) a first step the whole human mammary secretion is subjected to centrifugation leaving a fat layer on top, a protein and carbohydrate rich supernatant beneath it, and at the bottom a pellet of cells;

(ii) in a second step, the cell pellet is washed in media, preferably in RPMI media only

(iii) in a third step the cells of the cell pellet are plated onto a cell culture treated device in bacteriocidal and/or fungicidal growth media and are allowed to incubate, preferably for 10-30 days, most preferably for 14-20 days,

(iv) the cells are harvested, preferably by trypsinization, and washed, preferably using growth media

(v) the harvested cells are plated onto a reconstituted basement membrane preparation for growth preferably up to confluence.

17. A method according to claim 16, wherein in step (v) a solubilized basement membrane preparation extracted from EHS mouse sarcoma is used, as e.g. Matrigel™.

18. Progenitor cells, preferentially pluripotent or multipotent progenitor cells, derived using a method according to any of the preceding claims 1 through 17.
- 5 19. Use of pluripotent or multipotent progenitor cells as derived using a method according to any of the claims 1-17 for ex vivo, in vitro and/or in vivo applications.
- 10 20. Use according to claim 19, to create tissues or cells for the benefit of the mother and/or of the infant and/or of other individuals.
21. Use according to claim 17 or 20, including subsequent gene therapy treatments or intrauterine foetal treatments.
- 15 22. Use according to claim 19-21, for the generation of cells, tissue, glands or organs for the treatment of disease.
- 20 23. Use according to any of the claims 19-23, for subsequent cloning or scientific research.
24. Use according to any of the claims 19-23, for one or several of the group of the following purposes: clinical, diagnostic, bioengineering, lactoengineering, breast tissue regeneration, breast reconstructive surgery, breast cosmetic or enhancement surgery, exocrine gland tissue regeneration and/or surgery.